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The yeast mitochondrial intron aI5α: associated endonuclease activity and in vivo mobility

(Group-I introns; intron-encoded protein; mobile introns; restriction enzyme I-*Sce*IV; *Saccharomyces cerevisiae*)

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SUMMARY

By analyzing crosses between yeast strains carrying different combinations of mitochondrial (mt) introns, we have shown that the aI5α intron is mobile in vivo. Furthermore, we have observed that the mobility of intron aI5α is affected by both the nuclear and mt genotypes. We have also detected a restriction endonuclease (ENase) activity that cleaves intronless mt genomes close to the aI5α intron insertion site and thus might be involved in intron mobility. This is further supported by the fact that this ENase activity is only detected in a strain containing the aI5α intron. Furthermore, similar to other ENases encoded by mobile mt introns of yeast, the ENase generates a cut with a four-base 3'-OH overhang. Thus, intron aI5α represents a characteristic member of the family of mobile group-I introns.

INTRODUCTION

Several years ago the yeast mt RNA ω intron was shown to be mobile. Indeed, crosses between intron-less and intron-containing strains result in efficient transfer of the ω intron to genomes lacking it. This results in a pattern of inheritance in which nearly all descendent mt genomes contain the intron and also in enhanced transmission of the markers flanking it (recombination polarity) (reviewed in Dujon, 1981). Genetic experiments have demonstrated that a protein encoded by the ω intron itself is required for intron mobility (Jacquier and Dujon, 1985; Macreadie et al., 1985). Furthermore, this protein has ENase activity and cleaves mt genomes which lack the ω intron at the intron insertion site (Colleaux et al., 1986).

More recently, the yeast mt aI4 intron (Wenzlau et al., 1989), the rRNA intron of *Physarum polycephalum* (Muscarella and Vogt, 1989), the rRNA intron of *Chlamydomonas eugametos* chloroplasts (Lemieux and Lee, 1987), the COB intron of *Chlamydomonas smithii* mitochondria (Colleaux et al., 1990) and the *td* and *sunY* introns of phage T4 (Quirk et al., 1989) have been shown to be mobile. Furthermore, in the case of the yeast aI4 intron (Wenzlau et al., 1989; Delahodde et al., 1989), the *Physarum* rRNA intron (Muscarella and Vogt, 1989), *Chlamydomonas eugametos* rRNA intron (Gauthier et al., 1991) and the T4 *td* and *sunY* introns (Quirk et al., 1989; Beli-Pedersen et al., 1990), biochemical and/or genetic analyses have shown that the protein encoded within the intron is a site-specific DNA ENase cleaving the genome of the intron-less strain at or near the intron insertion point. An ENase encoded by the yeast mt RF3 reading frame (Séraphin et al., 1987; Nakagawa et al., 1991) and an ENase activity cleaving close to the intron aI3 insertion site have also been described (Sargueil et al., 1991).

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Abbreviations: bp, base pair(s); Δ, deletion; ENase, restriction endonuclease; kb, kilobase(s) or 1000 bp; mt, mitochondrial; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; r, ribosomal; wt, wild type.

The possibility that some of the other optional mt group-I introns are also mobile was tested previously by analyzing the descendents of crosses between long (intron-containing) and short (intron-less) strains (Zinn and Butow, 1984). However, no new mobile introns were uncovered. We noted that these experiments might have failed because exonic sequences flanking the optional introns differ. Such differences have been observed in the exonic sequences bordering the optional *COX1* introns aI5α and aI5β (Hensgens et al., 1983). To test whether aI5α or aI5β are mobile introns, we constructed a set of strains whose mt genomes are identical except for the presence or absence of the aI5α, aI5β and/or aI5γ intron(s). Analyses of crosses between these strains and a strain containing a complete set of introns revealed that intron aI5α is mobile. Further experiments suggest that the aI5α intron encodes a DNA ENase that cleaves the intron-less mt genome close to the intron insertion site.

RESULTS AND DISCUSSION

(a) In vivo mobility of intron aI5α

We first constructed a set of isonuclear and isomitochondrial strains containing various combinations of the aI5α, aI5β and aI5γ introns. For this purpose, we made use of the mss18 mutation that prevents efficient RNA splicing of intron aI5β and allows selection of revertants deleted of intron(s) present in the aI5β region of the mt genome (Séraphin et al., 1988; Levra-Juillet et al., 1989). Starting with a strain containing the wt 777-3A mt genome we obtained three kinds of revertants: those deleted of intron aI5β alone (hereafter referred to as ΔaI5β), those deleted of both introns aI5β and aI5γ (ΔaI5β,γ) or those deleted of all three aI5α, β and γ introns (ΔaI5α,β,γ) (see Table I). We also used an isonuclear strain containing the D273-10B mt genome which naturally lacks introns aI5α and aI5β (Table I).

To test the possibility that intron aI5α and/or aI5β are mobile, we crossed strains BS108-8/B115 or BS108-8/R29 containing, respectively, the ΔaI5β and ΔaI5α,β,γ mt genome with strain BS110-1C/E1 containing the full set of introns in the 777-3A mt genome. As controls, we also crossed strain BS110-1C/E1 with two isonuclear strains carrying either the 777-3A or the D273-10B mt genome. The mt DNA structure of the resulting diploid population was then analysed by isolating total DNA, cleaving it with *Bam*HI + *Pvu*II followed by Southern blotting using a probe covering exons aE5β, aE5γ and aE6. The results depicted in Fig. 1 can be summarized as follows: (*i*) both parental genomes (i.e., 777-3A and ΔaI5β) were recovered in similar amounts in cross BS108-8/B115 (ΔaI5β) with BS110-1C/E1 (all introns) (lane 2); (*ii*) the progeny of the cross BS108-8/R29 (ΔaI5α,β,γ) with BS110-1C/E1 (all introns) contain nearly exclusively the 777-3A mt genome with a full set of introns, although traces of both recombinants lacking only the aI5β and γ introns, and parental ΔaI5α,β,γ mt DNA could be detected (lane 4); (*iii*) following the cross BS108-1 (ΔaI5α,β from D273-10B) with BS110-1C/E1 (all introns) (lane 6) both the D273-10B and 777-3A parental mt genomes were recovered in similar amounts as reported previously (Zinn and Butow, 1984); (*iv*) as expected, only the 777-3A genome was found in diploids obtained by crossing strain BS108-8 (all introns) with strain BS110-1C/E1 (all introns) (lane 8).

TABLE I

Mitochondrial and nuclear genotype of *Saccharomyces cerevisiae* strains used in this study

Strains	Nuclear genotypes[a]	Mitochondrial genotypes[a]	References
BS110-1C	*MATa, ade1* or *ade2, his1*	777-3A	This work
BS110-1C/E1	*MATa, ade1* or *ade2, his1*	777-3A, E^R	This work
BS112-2C	*MATa, ade5, leu2*-112, *mss18*-3	777-3A	This work
BS108-1	*MATa, CAN*^{R}, *his3Δ1, leu2*-112, *mss18*-3	D273-10B	Séraphin et al. (1987)
BS108-8	*MATa, CAN*^{R}, *his3Δ1, leu2*-3, *leu2*-112, *mss18*-3	777-3A	Séraphin et al. (1987)
BS108-8/R29	*MATa, CAN*^{R}, *his3Δ1, leu2*-3, *leu2*-112, *mss18*-3	777-3A ΔaI5α,β,γ	This work
BS108-8/B115	*MATa, CAN*^{R}, *his3Δ1, leu2*-3, *leu2*-112, *mss18*-3	777-3A ΔaI5β	This work
BS108-8/R1	*MATa, CAN*^{R}, *his3Δ1, leu2*-3, *leu2*-112, *mss18*-3	777-3A ΔaI5β,γ	Séraphin et al. (1987)
BS104-8/R29	*MATa, CAN*^{R}, *his3Δ1, leu2*-3, *leu2*-112	777-3A ΔaI5α,β,γ	This work
MGD353-13D/2	*MATa, ade2, arg4, leu2-3, leu2*-112, *trp1*-289, *ura3*-52	rho*	This work
K[R29]	*MATa, CYH*^{R}, *kar1*-1, *trp5*	777-3A ΔaI5β,γ	This work
BSY208	*MATa, CAN*^{R}, *leu2*-3, *leu2*-112, *ura3*-52	777-3A ΔaI5β,γ	This work
BSY209	*MATa, CAN*^{R}, *leu2*-3, *leu2*-112, *ura3*-52, *mss18*-3	777-3A ΔaI5β,γ	This work
CWO4	*MATa, CAN*^{R}, *ade2*-1, *trp1*-1, *leu2*-3, *his3*-11	777-3A	Banroques et al. (1986)
CWO2	*MATa, CAN*^{R}, *ade2*-1, *trp1*-1, *leu2*-3, *his3*-11	777-3A Δbi4	Labouesse and Slonimski (1983)

[a] See Sherman (1981) for yeast genetic nomenclature. E^R, Erythromycin resistant.

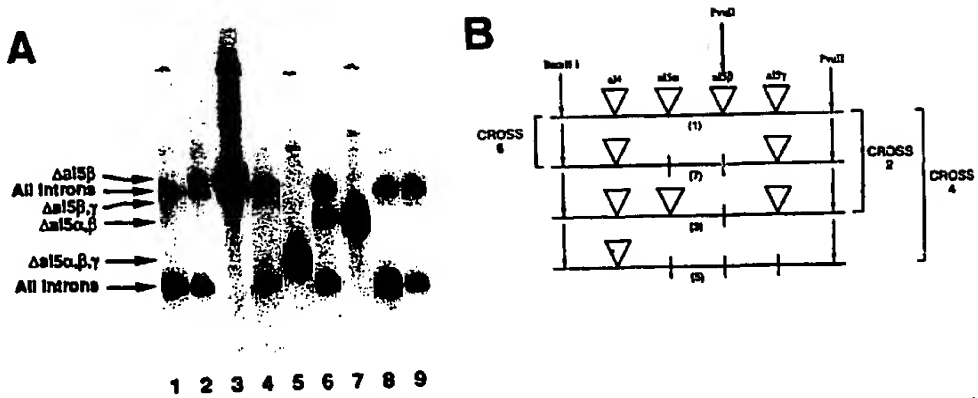

Fig. 1. Analyses of crosses following the strategy of Wenzlau et al. (1989). Southern blotting of *Bam*HI+*Pvu*II-digested DNA from either parental strains or diploid populations resulting from crosses is shown in panel A. The samples were run on 0.8% agarose gel. The ^{32}P-labelled probe used covers exon sequences from the *Nco*I site in exon aE5β to the *Eco*RI site in exon aE6. Intron combinations corresponding to each band are indicated in B. Two bands correspond to the structure containing all three al5 introns due to the presence of a *Pvu*II site in the al5β intron. Lane 1, BS110-1C/E1 (all introns); lane 2, BS110-1C/E1 × BS108-8/B115; lane 3, BS108-8/B115 (Δal5β); lane 4, BS110-1C/E1 × BS108-8/R29; lane 5, BS108-8/R29 (Δal5α,β,γ); lane 6, BS110-1C/E1 × BS108-1; lane 7, BS108-1 (Δal5α,β from D273-10B); lane 8, BS110-1C/E1 × BS108-8; lane 9, BS108-8 (all introns). Schematic physical maps of the different alleles are shown in panel B. Inverted triangles indicate the presence of introns. Numbers refer to the lanes of the adjacent gel (in panel A); they indicate the lanes corresponding either to the parental strains (numbers in parentheses) or to the different crosses (side brackets and cross).

Quantitative data were obtained for crosses BS108-8/B115 (Δal5β) with BS110-1C/E1 (all introns) and BS108-8/R29 (Δal5α,β,γ) with BS110-1C/E1 (all introns) by analyzing the mt DNA structure in individual diploid subclones. All of the 18 diploids from the latter cross contained a full set of introns while this was true for only eight diploids out of 14 in the former case.

These results suggest that intron al5β is not mobile. However, we can not exclude the possibility that this intron is mobile under different conditions. On the other hand, the result from cross BS108-8/R29 with BS110-1C/E1 suggests that either some of the three introns al5α,β or γ are mobile or that there is a preferential transmission of the w+ genome in this particular cross. This latter possibility seemed unlikely because the crosses analyzed were isonuclear and isomitochondrial. Furthermore, a similar ratio of E^R to E^S (see Sherman, 1981, for genetic nomenclature) diploids was found in the four crosses analyzed, indicating that the various intron combinations did not affect the transmission of the different mt genomes. Another possibility might have been that the nearly exclusive recovery of the full-length mt genome in cross BS108-8/R29 with BS110-1C/E1 resulted from some respiratory disadvantage conferred by the Δal5α,β,γ genome. This was rendered unlikely, however, because the 777-3A mt genome was still recovered nearly exclusively when crosses and analyses were performed in the presence of a high concentration of glucose (data not shown).

Thus, we were left with the conclusion that at least one among the three al5 introns was mobile. The most likely candidate was intron al5α because it is the only one of these three introns to be present in the Δal5β,γ recombinant mt genome that we detected in cross BS108-8/R29 with BS110-1C/E. This hypothesis was also supported by our data showing that intron al5β is not mobile by itself under identical conditions (see this section above) and by the fact that intron al5γ is unlikely to be mobile because it does not have the capacity to code for a protein involved in intron mobility. Although intron al5β and al5γ were unlikely to be mobile on their own, it was not unexpected to find them in most of the progeny of cross BS108-8/R29 with BS110-1C/E1 because the transfer of the al5α intron to the intron-less genome was likely to induce conversion of the polymorphic markers flanking intron al5α, including introns al5β and al5γ. We noted, however, that intron al5α was not mobile in cross BS108-1 (Δal5α,β from D273) by BS110-1C/E1 (all introns), suggesting that the D273-10B mt genome (which, unlike the others, is not derived from strain 777-3A) was not a recipient for intron transposition (see section e).

To confirm that intron al5α was mobile, we constructed strains of the opposite mating-type whose mt genomes differed only by the presence or absence of the al5α intron. We then analyzed the structure of the mt genome in the progeny of crosses between strains containing the Δal5β,γ mt genome and strains containing the Δal5α,β,γ genome. In all eight different crosses analyzed (Fig. 2 and data not shown) the al5α-containing mt genome (Δal5β,γ) was found nearly exclusively in the diploid progeny (lanes 3 and 4) with only traces of the intron-less genome from the other parent. This result confirmed unambiguously that intron al5α is mobile.

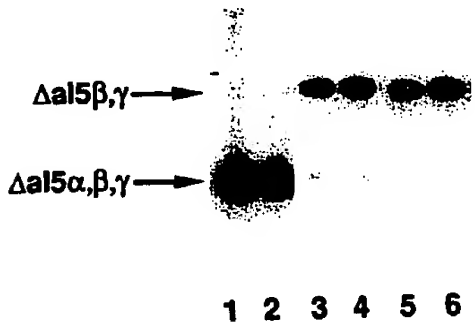

Fig. 2. Mobility of the al5α intron. Intron content of the mt DNA in strains BS104-8/R26 (Δal5α,β,γ; lane 1), BS108-8/R29 (Δal5α,β,γ; lane 2), BSY209 (Δal5β,γ; lane 5) and BSY208 (Δal5β,γ; lane 6). Lanes 3 and 4 depict the intron content of the mt DNA in the diploid population resulting from crosses BS108-8/R29 with BSY209 and BS104-8/R29 with BSY208, respectively. Intron combinations corresponding to each band are indicated on the left. Analyses follow the strategy described for Fig. 1.

(b) Mobility of the al5α intron is influenced by the nuclear genotype

While the preceding results show that intron al5α present in the 777-3A mt genome is mobile, we serendipitously discovered that this mobility is strongly influenced by the nuclear genotype. Fig. 3 presents the results of the analyses of crosses between strain BS108-8/R29 and either strain BS110-1C or strain BS112-2C. The former strain contains the Δal5α,β,γ mt genome while the latter ones are both isomitochondrial with strain 777-3A. As described above, the diploid progeny of cross BS108-8/R29 with BS110-1C contain nearly exclusively the 777-3A mt genome and only traces of the parental Δal5α,β,γ and recombinant Δal5β,γ genomes. On the other hand, the progeny of the cross BS108-8/R29 with BS112-2C contain approximately similar amounts of each of the same three mt genomes (Fig. 3, lane 4). These results suggest that the transfer of intron al5α to the intronless mt genome occurred inefficiently in the cross BS108-8/R29 with BS112-2C. Nevertheless, the presence of recombinant Δal5β,γ genome indicates that some intron transfer did occur.

The possibility that the reduced transmission of the al5α intron was related to the presence of the mss18-3 allele in a homozygous state was excluded by the following observations: (*i*) the same effect was observed using wt MSS18+ strains instead of BS112-2C (mss18-3); (*ii*) this effect was not observed when using other strains also containing the mss18-3 allele but otherwise unrelated to BS112-2C (e.g., Fig. 2 and data not shown). Furthermore, the same results were obtained when the same strains were grown in the presence of a high concentration of glucose indicating that this effect is unlikely to result from some selection pressure on the respiratory competent cells, but rather reflects some

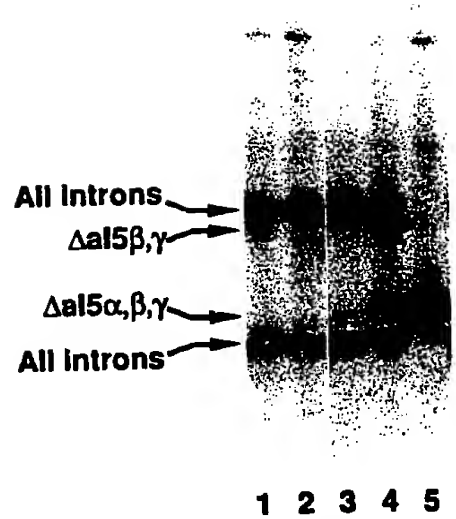

Fig. 3. Nuclear genotype affects intron al5α mobility. Intron content of the mt DNA in strains BS110-1C (all introns; lane 1), BS112-2C (all introns; lane 2) and BS108-8/R29 (Δal5α,β,γ; lane 5). Lanes 3 and 4 depict the structure of the mt DNA in the diploid population resulting from crosses of BS108-8/R29 with BS110-1C and BS112-2C, respectively. Intron combinations corresponding to each band are indicated on the left of the figure. Analyses follow the strategy described for Fig. 1.

difference in the nuclear genotype of strains BS110-1C and BS112-2C.

What might be the role of nuclear gene(s) in intron al5α mobility? It is possible that some of these nuclear genes could encode components of the mt recombination apparatus. Alternatively, nuclear genes could control the synthesis or activity of mitochondrially encoded proteins, such as ENases, involved in intron mobility. In fact, the recent discovery (Nakagawa et al., 1991) that a mt DNA ENase can be composed of both a mitochondrially and nuclearly encoded product strengthens this hypothesis. Further work is required to identify more precisely such gene(s) and to pinpoint its(their) role(s) in intron al5α mobility.

(c) ENase activity cleaves intron-less mt genomes close to the al5α intron insertion site

Because group-I intron mobility in yeast mitochondria is associated with the presence of ENase cleaving the intronless mt genome close to the intron insertion site, we looked for such an activity in the case of intron al5α. We followed an approach which already allowed us to detect two other DNA ENase activities (Sargueil et al., 1990; 1991) in the wt cell. Because of the low level of intronic proteins in such a genetic context the cleavage products can be difficult to detect. For this purpose, we first synthesized oligos covering the 29-bp sequence of the al5α insertion site in the strain 777-3A (Fig. 5) and inserted them into the pUC19 plasmid. We then assayed for the cleavage of the linear

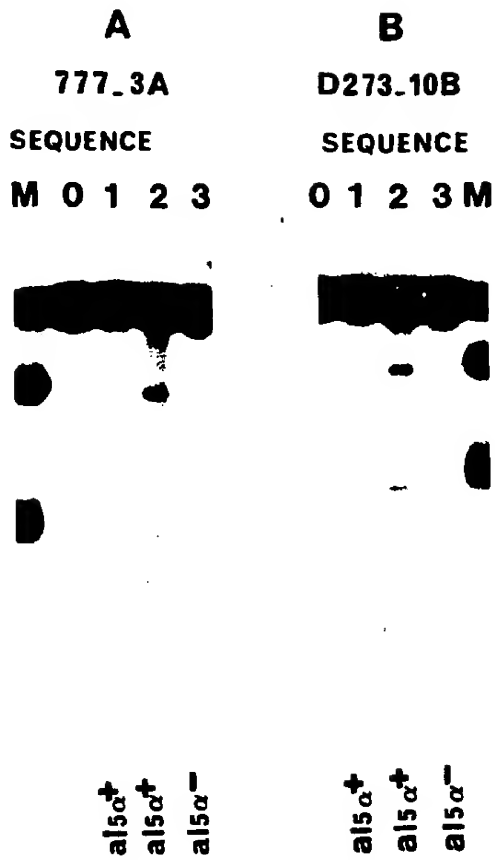

Fig. 4. Mitochondrial extracts from al5α-intron-containing strains possess an ENase activity which cleaves recipient DNA near the site of intron insertion. Recipient DNAs to test the DNA ENase activity were prepared as follows: the oligos 5'-GATCCATTTTCTCATGATTAGCTCTAATCCATGGCTGCA and 5'-GCCATGGATTAGAGCTAATCATGAGAAATG representing the exonic flanking sequences of the al5α intron in the mt 777-3A, were synthesized, hybridized and cloned between the *Bam*HI and *Pst*I sites of vector pUC19. A similar vector was constructed with the sequence corresponding to the strain D273-10B (Fig. 5). The mt extracts from the different strains were prepared as previously described (Sargueil et al., 1990) and similar amounts of proteins (15–20 μg) were added to the recombinant plasmid previously linearized by a digestion with *Sca*I. Incubation conditions were as in Wenzlau et al. (1989). Two different recipient DNAs corresponding to the strain 777-3A (panel A) and D273-10B (panel B) were used. The mt extracts from the strain CW04 (lane 1) and from two isogenic strains [BS108-8/R1 (intron$^+$, lane 2) and BS108-4/R29 (intron$^-$, lane 3)] were assayed for their ability to cleave the recipient DNA. Southern blots were hybridized with a ^{32}P-labeled nick-translated recombinant plasmid. Lane O corresponds to the linear recombinant plasmid alone. Lane M contains the digestion products of the al4 DNA ENase on a recipient DNA containing the al4 insertion site (Delahodde et al., 1989). Sizes of fragments in lane 2 (1.8 kb and 0.92 kb) compared to those in lane M correspond to a cut in the vicinity of the al5α insertion site. Analysis was done on 0.8% agarose gel.

form of this recombinant plasmid in the presence of mt extracts derived from a strain containing the al5α intron. Results shown in Fig. 4 indicate that an ENase activity can readily be detected using this assay.

Because we did not detect intron al5α mobility using the D273-10B mt genome as a recipient, we next asked if the ENase activity that we had detected was able to cleave the same region of the D273-10B mt DNA. Indeed, as indicated above, the nt sequence around the al5α intron insertion site differs somewhat between strains D273-10B and 777-3A (Fig. 5). Oligos corresponding to the D273-10B sequence were then synthesized and incubated in mt extracts. Surprisingly, we found that the ENase activity was able to cleave the sequence containing the D273-10B aE5α-aE5β junction as well as the 777-3A junction. This is reminiscent of our observations in the case of the al4 DNA ENase (I-*Sce*II) which tolerates nt changes in the target site especially when they are located in the third nt position (Sargueil et al., 1990). This property could be related to the horizontal transfer capacity of group-I introns (Belfort, 1991). The apparent ability of the al5α DNA ENase to cleave different DNA sequences makes the absence of intron insertion in the D273-10B genome more puzzling. A likely interpretation is that the ENase display an altered specificity in vitro. However, we cannot exclude that polymorphism at other locations in the mt genome prevent the D273-10B genome from acting as an acceptor. Another possibility is that the D273-10B mt DNA might be protected from cleavage in vivo but not in vitro. Indeed, the sequence present around the al5α intron insertion site in strain D273-10B might allow the binding of a factor that blocks ENase attack. Sequence variation would however prevent binding of this factor on the homologous region of the 777-3A mt genome.

(d) Mapping the cleavage site

We then decided to determine the precise point of cleavage introduced by this ENase. Because of the scarcity of the cleavage product we had to develop a new approach. Analysis of the cleavage site was conducted on the recombinant plasmid linearized by the ENase and purified by agarose gel electrophoresis. We first delineated the cleavage site by labelling DNA fragments containing the ends with T4 DNA polymerase and limiting amounts of the four radioactive dNTPs (Delahodde, 1988). These preliminary studies were confirmed by an experiment in which T4 DNA polymerase-treated plasmid was self-ligated before amplification and DNA sequencing. We recovered several independent clones containing a 4 bp deletion (Fig. 5). This demonstrates that the al5α-dependent ENase generates a four base 3'-OH overhang.

(e) The protein encoded within intron al5α is translated as a fusion protein and controls ENase activity

By analogy with the other mobile introns, we decided to test if the ENase cleaving near the intron al5α insertion site is also encoded by intron al5α itself. For this purpose, we

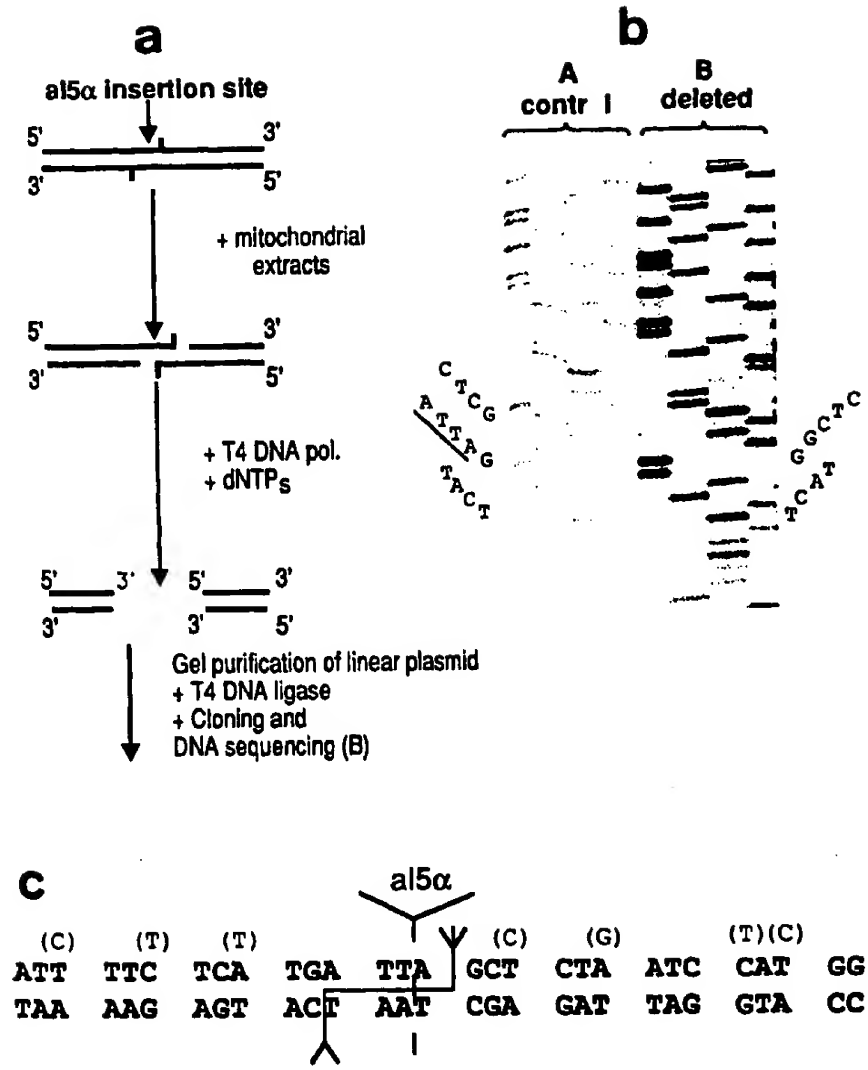

Fig. 5. Cleavage site of the al5α-dependent DNA ENase activity. Map a in this figure depicts the strategy followed to analyze the cleavage site. Briefly, the circular recombinant plasmid was incubated with mt extracts under standard conditions and the digested linear plasmid was purified on agarose gel, extracted and then incubated with T4 DNA polymerase with an excess of dNTPs as described by Challberg and Englund (1980). DNA ligation, *E. coli* transformation and nt sequencing allowed establishment of the cleavage site (see part B, panel b). In panel b, the nt sequence of the cleaved and treated plasmid is shown in B alongside the corresponding sequencing gel. For comparison, the corresponding data for the untreated plasmid are depicted in A. Map c shows the sequence of strain 777-3A (lower lines) in the vicinity of the al5α insertion site (indicated). KL14-4A (Hensgens et al., 1983) and 777-3A have identical sequences in this region (our unpublished results). The nt in parentheses correspond to the D273-10B sequence (Bonitz et al., 1980).

prepared mt extracts from two isonuclear and isomitochondrial strains differing only by the presence or absence of intron al5α (namely BS108-8/R1 and BS108-8/R29). We then assayed cleavage of the 777-3A oligo in these extracts. As depicted in Fig. 4, oligo cleavage was detected only in the extract derived from the al5α-containing strain but not in extract derived from the al5α-less strain. This result suggests that the ENase activity detected is encoded by the al5α intron itself, because it is the only polymorphic marker differing between the two strains.

In a second experiment we asked if removal of intron al4 was prerequisite for ENase synthesis. The reading frame present in intron al5α is in frame with the upstream exons, and thus its translation should be dependent upon prior removal of upstream introns. To test this hypothesis, we prepared mt extracts from the strain CWO2 which is deleted for the bI4 intron. In such a strain the bI4 RNA maturase is not made and the RNA splicing of the aI4 intron is thus blocked (Labouesse and Slonimski, 1983). When tested for their DNA ENase activities, CWO2 mt extracts did not reveal any al5α ENase activity whereas they contain considerable al4 DNA ENase activity (Fig. 6).

Fig. 6. Block in the splicing of the upstream intron alters the al5α dependent DNA ENase activity. ENase activities of mt extracts were examined as indicated previously (Fig. 4). Recipient DNAs contained either the 29-bp al5α insertion site (al5α/IS, lanes 0 and 2) or the al4 insertion site (Delahodde et al., 1989, al4/IS, lanes 1). The mt extracts from wt mitochondria (CW04, W^+, panel A) or from mutated mitochondria (CW02, Δbl4, panel B) were assayed on the two types of recipient DNA. The CW02 strain has no bI4 RNA maturase activity which is required for splicing of the al4 intron. No mt extracts were added in lane 0.

This suggests that splicing of intron al4 is required for the al5α ENase synthesis and that this ENase, like many other intronic proteins, is translated from a fused exon-intron *COX1* sequence.

(f) Conclusions

(*1*) Our results show the existence of an ENase that cleaves close to the al5α intron insertion site. The existence of similar ENases has been reported for other mobile group-I introns. In such cases the ENase has been shown to be encoded by the mobile intron itself. This seems to be true for intron al5α as well since deletion of intron al5α abolishes the ENase production. Although we cannot exclude that the intron al5α-encoded protein is an accessory factor in the DNA ENase activity, we favour the possibility that it is itself the ENase because of its high similarity with other group-I intron-encoded proteins. Such as possibility is strengthened by our observation that the al5α cleavage site is similar to those of I-*Sce*I (Colleaux et al., 1988) and I-*Sce*II (Wenzlau et al., 1989; Delahodde et al., 1989) with a 4-nt overhanging 3′ sequence in the vicinity of the intron insertion site (Fig. 5). From the presented observations and according to previous suggestions (Dujon et al., 1989), we propose to name this new intronic activity I-*Sce*IV.

(*2*) Consistent with our detection of an ENase activity that cleaves close to the intron al5α insertion site, we demonstrated that intron al5α is mobile in vivo. Thus, intron al5α belongs to the ever-growing family of mobile group-I introns.

(*3*) Analysis of iso-mt crosses revealed that mobility of intron al5α is dramatically affected by nuclear genotype. A further level of control of intron al5α mobility resides in the mt genome itself. Our results suggest an intricate nucleo-mt control of intron al5α mobility. One should keep in mind that such mechanism(s) might hamper intron movement and further suggest that mobile group-I introns might be more prevalent than previously thought.

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